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which fluoresce upon intercalation into amplification products but need an excitation source and electronic detection system.

Example 2

Prototype Device and Assay

A Point-Of-Care prototype device and assay have been designed, which encompass sample acquisition, sample preparation/nucleic acid extraction, LAMP/RT-LAMP amplification, and detection in a single tube. A rendering of the prototype device is shown in FIGS. 3A-3B. The device includes a sealable polypropylene tube with a loading port, a hollow polyester swab coupled to a tube cap, a 4 mm disc of cellulose FTA card (Whatmann®) positioned within the tube, and reagents including dye for conducting sample preparation such as nucleic acid purification and RT-LAMP. The swab facilitates sample acquisition from surfaces, oral or nasal cavities, or lesions.

A procedural flow chart of performing the assay using the Point-Of-Care device is shown FIG. 4C. First, the cap with hollow swab is removed from the tube (Step 4C.1). Then, a Sample is collected by wiping material of interest with the hollow swab (Step 4C.2). After sample collection, the swab is returned to the tube, and the tube cap is secured in a manner that precludes re-opening of the tube (step 4C.3). A first syringe containing 4 ml of Purification Reagent (Whatman®) is attached to a luer lock (308) fitting on the cap (307) (Step 4C.4), and then 2 ml of the Purification Reagent is delivered through the swab lumen to immerse the sample in a purification wash (Step 4C.5). The tube is swirled and set in a rack for approximately 2 min, followed by waste removal through the tube's lower loading port (for example, a septum) (305) via a pipette (Step 4C.6). Purification Reagent steps then are repeated.

The first syringe is decoupled from the luer lock, a second syringe containing 4 ml TE buffer is attached, and 2 ml TE buffer is delivered through the swab lumen to rinse the sample (Step 4B.7). After swirling of the tube and about 2 min of waiting, waste is removed (Step 4B.8), and the TE buffer steps are repeated. At this point, sample has been transferred from the swab to the FTA disc and cleaned.

Next, LAMP/reverse transcriptase (RT-LAMP) master mix plus enzymes are pipetted into the evacuated tube through the loading port (Step 4C.9). One or more assay tubes then are placed on the custom thermal heater rack (FIG. 3B), which is maintained at an isothermal temperature of about 63° C. for 45 min to 1 hr., to conduct RT-LAMP amplification (Step 4C.10). Positive amplification is determined by a visible color change in the reagents from purple to blue, due to the presence of colorimetric dye (Step 4C.11).

Example 3

Detection Under BSL3 Condition for Serotype O Foot and Mouth Disease Virus (FMDv)

Proof-of-concept validation of the Point-of-care methods and apparatus according to the current disclosure has been conducted by amplifying and detecting serotype O Foot-and-Mouth Disease virus (FMDv). Foot-and-Mouth Disease is a highly infectious viral disease of cloven-hoofed animals, including cows, sheep, goats and pigs. It is one of the most infectious diseases that affect livestock, and outbreaks can spread quickly. The virus can survive in contaminated fodder and the environment for up to one month and can survive in

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the lymph nodes and bone marrow of infected animals. There are at least seven different types and many subtypes of the FMD virus, the most common of which is serotype O (see FIG. 2).

Humans can spread FMDv through contaminated clothing, footwear, farm equipment and trucks. It also can spread through the air from one animal to another. Most commonly, it spreads through a contaminated source of water, hay or feed. FMDv epidemics have resulted in the slaughter of millions of animals, despite the fact that, frequently, it is non-fatal. Culling is primarily performed to halt disease spread, as growth and milk production may be permanently affected in recovered animals. Infection also may lead to trade bans on affected countries.

The UK FMD epizootic in September of 2001 led to a \$17 billion cost to the economy. 2,030 farms were infected; 15,000 antigen tests, and more than 1 million ELISA tests, were performed. Average time from field collection to receipt at a centralized laboratory was 1.2 days, so many decisions were based on clinical diagnosis without laboratory confirmation. No evidence was found in 23% of all farms declared infected.

Clearly, FMDv has substantial relevance to veterinary medicine and represents a virus with enormous economic impact that would benefit from in-the-field POCT in accordance with the prototype device (Ferris et al., 2006, Vet. Rec., Vol. 159, pp 373-378).

Point-of-care testing on recombinant FMDv, and clinical isolate tissue homogenate (live virus, BSL3, Pirbright, UK) was performed. FIG. 8 shows exemplary images of negative, no template controls (NTC) and positive, template controls run with recombinant template at the start and at 45 min. Electrophoresis gels were run on 2 µL aliquots removed from the loading port to confirm colorimetric results (FIG. 11A). FIG. 9 shows images of a dilution series of live FMDV taken at start and 60 min. A gel was run to confirm the colorimetric results (FIG. 11B). The results indicate that, while performing the entire FMDV assay in a single disposable tube, we can colorimetrically detect virus diluted down to 10⁻⁵ dilution in about 45 min. Parallel testing of these samples (in triplicate) by real-time RT-PCR generated C_T values (±standard deviation) of 15.7±0.1, 19.6±0.3, and 31.0±0.1 for the 10⁻¹, 10⁻², and 10⁻⁵ dilutions, respectively.

FIG. 10 shows colorimetric detection of recombinant FMDv after 30 min. Top panel indicates initial color of HNB dye in 25 mL reactions covered with a drop of mineral oil. Middle panel shows a shift from purple to blue by 30 min in tubes 3 and 4, which contained 5 mL template material (tubes 1 and 2 were no template controls). Lower panel compares colorimetric detection upon adding dye at 30 min timepoint, as opposed to incorporating it at reaction initiation (first 3 tubes no template; tubes 4 and 5 contained 5 mL template).

Example 4

Detection Under BSL1 Condition for MRSA Genomic DNA

FIG. 12 shows time series images of MRSA assay in prototype tubes. Genomic DNA transferred from swabs to filter paper during sample preparation and amplification was performed on material embedded in the paper. FIG. 13 shows a 45-min MRSA assay in microcentrifuge tubes in order to assess preliminary assay sensitivity. Data indicate that the colorimetric assay can detect ~17 genomic copies of MRSA. Water served as no template control (NTC). Four percent